Supporting Information Appendix: A Growing Microcolony can Survive and Support Persistent Propagation of Virulent Phages

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1 Simulation method

1.1 Detailed protocol of the numerical simulation

Cell growth and cell-cell interaction

We constructed a 3-dimensional model of cells and surrounding phage particles. The cell are labeled by an index i and modeled as spheres with radius r_i, a position $\vec{x}_i$, and a state S_i. The cell experiences a repulsive potential V_{ij} from every other cell j of the form $V_{ij}(d_{ij}) = \frac{\tilde{k}}{2}\left(\frac{d_{ij}-r_i-r_j}{r_i+r_j}\right)^2$ if $d_{ij} \leq r_i + r_j$. The harmonic potential is chosen as it is the simplest repulsive potential and it has been used previously to model cell-cell interaction [1]. Here $\vec{d}_{ij} = \vec{x_i} - \vec{x_j}$ and $d_{ij} = ||\vec{d}_{ij}||$ is the euclidean distance between the cells. $\tilde{k}$ parametrizes the repulsion strength. The potential is set to be zero when $d_{ij} > r_i + r_j$. The motion of cells is computed in the over-damped limit ($m\ddot{x} \approx 0$), leading to the equation of motion: $\dot{\vec{x}}_i = -\frac{1}{\mu}\sum_{j\neq i}\frac{\mathrm{d}}{\mathrm{d}d_{ij}}V_{ij}\hat{\vec{d}}_{ij}$, where $\hat{\vec{d}}_{ij}$ is the unit vector parallel to $\vec{d}_{ij}$ and μ is a viscosity parameter. This viscosity and the repulsion strength $\tilde{k}$ can be absorbed together to a single variable k.

Cell growth follows a Monod growth, $\dot{r_i} = \frac{g(n)}{3}r_i$, where the nutrient-dependent growth rate $g(n) = g_{\max}\frac{n}{n+K}$. The maximal growth rate $g_{\max}$ is taken to be $\frac{1}{30\ \mathrm{min}}$ which corresponds to a doubling time of approximately 20 minutes. The Michelis-Menten constant was chosen to be $K = n(t=0)/5$.

Nutrient depletion

For simplicity, we only allow uninfected cells to grow and consume nutrients. We further assume that nutrients diffuse infinitely fast and are not hindered by the presence of the cells. Hence there is no spatial dependency in the growth rate. We confirmed that the relevant range of colony sizes was small enough that the simulation with a finite diffusion rate of nutrient with realistic parameters did not deviate noticeably from our infinite diffusion simulation. The depletion of nutrients takes the form $\dot{n} = -\frac{g(n)}{V}\sum_i \delta_{S_i,0}$, where state variable $S_i = 0$ denotes that the cell i is uninfected by phage, δ is the Kronecker delta function, and V is the volume of the system.

Cell Division

Each cell grows until it reaches the volume 2 $\mu\mathrm{m}^3$ (or $R_d = 0.782$ μm) where it divides. The choice for R_d is such that the median cell volume in the simulation is 1.3 $\mu\mathrm{m}^3$. During division, the parent cell p is removed and two daughter cells are placed close to the center of the parent cell with some randomness $x_i^{(j)} = x_p^{(j)} + \zeta$, where $i = (1,2)$ refer to the daughter cells, (j) denotes the j'th component of the position $\vec{x}$ and ζ is a uniformly distributed number in $[-\frac{R_d}{4}, \frac{R_d}{4}]$. One daughter cell receives the fraction $\alpha \sim 0.5$ of the parents volume, while the other receives the remaining fraction: $r_1 = r_p\alpha^{\frac{1}{3}}$ and $r_2 = r_p(1-\alpha)^{\frac{1}{3}}$. Here noise is introduced through the random number α that is drawn from the Normal distribution with average 0.5 and standard deviation 0.1. Noise in the cell position and the cell size is introduced to avoid complete synchronization of the cell divisions as well as an artificially regular packing of the cells [2].

Phages

The phages are treated as individual point particles described with positions $\vec{y}$. The phages diffuse in the simulation volume, following the over-damped Langevin equation: $\dot{y}_k^{(j)} = \vec{F}_k(y_k^{(j)}(t)) + \xi(t)$, where $y_k^{(j)}$ is the jth-component of the position of the k'th phage; $\xi(t)$ is a noise term with a Gaussian probability distribution: $\langle\xi(t)\rangle = 0$ and $\langle\xi(t),\xi(t')\rangle = 2D_p\delta(t-t')$. There is no phage-phage interaction and when there is no cell-phage collisions the force term vanishes $\vec{F}_k = 0$. However, when a phage collides with a cell it is exposed to a repulsive force described by the interaction potential $V_k(d_{ki}) = \frac{\tilde{k}}{2}\left(\frac{d_{ki}-r_i}{r_i}\right)^2$ if $d_{ki} \leq r_i$, where d_{ki} is the euclidean distance between the k'th phage and the i'th cell with radius r_i. Furthermore, we include phage decay at a rate δ. The simulation was done in a cube of volume V with a reflective boundary conditions for the phages.

Cell-Phage interaction

Collision between phage k and cell i is defined as $d_{ki} \leq r_i$. The infection of the i'th cell by the k'th phage occur at a rate γ as long as the phage is within the cell radius. When the cell is infected by the phage, the state S_i is set to $S_i = 1$ if the cell was previously in the uninfected state ($S_i = 0$), otherwise the state is unchanged. To take into account the time delay before cell lysis, we use a ten-step Poisson process [3]. It starts when a cell is first infected and we increase the cell state S_i at a rate $10/\tau_L$ until the state is $S_i = 11$, where lysis occurs and β new phages are spawned uniformly around the cell's center and the cell is removed. We assume τ_L is inversely

proportional to the host growth rate $g(n)$ as $\tau_L = 1/(rg(n))$ to reflect the phage reproduction dependence on the host metabolism [3].

$\gamma = \infty$ describes the diffusion limited adsorption of phage. The phage adsorption rate η in Fig. 3D in main text was adjusted by changing γ, where η for a given γ was measured by simulating adsorption to a single cell in a limited volume.

Simulation initialization

The simulation starts with a single cell of volume 1.3 μm^3, which is allowed to establish a colony for T_i time, after which the phages are added. At time T_i, the geometric center of the colony is computed $x_{gc}^{(j)} = \frac{1}{N}\sum_i x_i^{(j)}$, where N is the number of cells at time T_i. The maximal distance of any cell to the geometric center $r_{\max} = \max_i ||\vec{x_i} - \vec{x_{gc}}||$ is computed. Then $P_0 \cdot V$ phages are spawned uniformly in the simulation space outside the sphere defined by position $\vec{x_{gc}}$ and radius $r_{\max}$.

Time Integration

For the presented data here, the model was implemented using the explicit Euler method with a time-step of $\Delta t = 10^{-6}$. The consistency of the integration method were confirmed by checking the results are the same with different time-step and with different integration methods (the standard fourth-order Runge-Kutta method and the fifth order Runge-Kutta-Dormand-Prince method were tested for solving the deterministic part of the equations) at a few selected parameter sets.

1.2 Simulation parameters

Table S1: Summary of the default parameters. These values are used unless otherwise mentioned. The values are chosen to be applicable for phage P1 parameters when available.

Name	Value	Units	Description	Comments / References
ΔT	10^{-6}	h	Size of the time step	
L_{box}	65	μm	Side length of simulation volume	
n_0	0.5	$1/\mu$m^3	Initial concentration of nutrient	
g_{max}	2	1/h	Maximal growth rate for the cells	Based on liquid culture conditions / [3]
K	$n_0/5$	$1/\mu$m^3	Michaels-Menten constant for Monod growth	Based on liquid culture conditions / [3]
R_d	0.782	μm	The length scale for division	
k	10^3	μm^2/h	Parameter for cell-cell interaction potential	
P_0	0.01	$1/\mu$m^3	Density of invading phage	
γ	∞ [1]	1/h	Phage infection rate for diffusion limited case	
	10^5 to 10^6	1/h	to reduce the phage adsorption rate	
β	400		Burst size	Based on liquid culture conditions / [4]
δ	0.003	1/h	Rate of phage decay	Based on liquid culture conditions / [4]
r	0.6		Constant for lysis latency time	Based on liquid culture conditions / [4]
D_P	$4 \cdot 10^3$	μm^2/h	Diffusion constant for the phage	1/10 of λ phage diffusion constant, estimate based on the small plaque size /[3]
D_n	$4 \cdot 10^5$	μm^2/h	Diffusion constant for the nutrient	Based on liquid culture conditions / [3] Used in supplement

1.3 Outline of simulation implementation

The algorithm of the simulation is shown as a pseudo-code in Algorithm 1 and goes as follows:

1. Phages infect bacteria
2. Infection stage is updated, and cells marked for bursting
3. Movement is calculated for phages and cells (but not executed)
4. Nutrients are consumed and cells are grown
5. The movement is executed, and phages decay, and cells burst

Algorithm 1 The update step

for all p_i **do** ▷ Loop over all phages in the system
  if PhageInfection(p_i) == 1 **then** ▷ PhageInfection returns 1 if phage successfully infected a cell
    p_i is deleted.
  end if
end for
for all c_i **do** ▷ Loop over all cells in the system
  GrowInfection(c_i) ▷ GrowInfection drives the infections forward if cell is infected
end for
for all c_i **do** ▷ Loop over all cells in the system
  CellMovement(c_i) ▷ Compute the movement of the cell
end for
for all p_i **do** ▷ Loop over all cells in the system
  PhageMovement(p_i) ▷ Compute the movement of the phage
end for
$n \leftarrow n - \frac{1}{V} g_{max} \cdot \frac{n}{n+K} \cdot \sum_{c_i} \delta_{S_i,0} \cdot \Delta T$ ▷ Consume nutrient
for all c_i **do** ▷ Loop over all cells in the system
  if $S_i == 0$ **then** ▷ Check if cell is uninfected
    GrowCell(c_i) ▷ Grow the cell and divide if radius increases above threshold
  end if
end for
for all c_i **do** ▷ Loop over all cells in the system
  if $S_i == 11$ **then** ▷ Check if cell is lysed
    c_i is deleted.
  else
    ApplyMovement(c_i) ▷ Execute the movement of the cells
  end if
end for
for all p_i **do** ▷ Loop over all phages in the system
  if $rand() \leq \delta \cdot \Delta T$ **then** ▷ Check if phage decays
    p_i is deleted.
  else
    ApplyMovement(p_i) ▷ Execute the movement of the phages
  end if
end for

2 Determining the adsorption rate

We determine the adsorption rate η as a function of the infection rate γ, by considering the free space diffusion of phages. This is achieved by using our simulation framework to measure the time it takes for a phage to hit and successfully adsorb to a small target. We construct a small spherical target of radius $R = 0.677\mu$m (median cell volume) inside a box of size $L^3_{box} = 30 \times 30 \times 30\mu m^3$. We then simulate the adsorption of 10000 phage particles, and measure the distribution of free phages as a function of time for every value of γ used in the article. Snapshots of the free phage distribution are shown in Fig. S1 for $\gamma = 10^6$ h^{-1}.

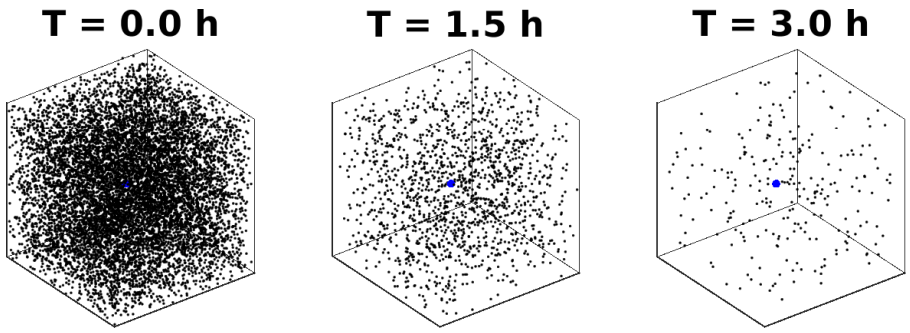

Figure S1: Snapshots of the adsorption simulation for $\gamma = 10^6$ h^{-1} at times $T = 0$ h, $T = 1.5$ h and $T = 3.0$ h. Each black dot represents a phage and the blue sphere represents the phage target.

The distribution of the number of free phages can be measured against T/L^3_{box} (blue points in Fig. S2 (left)). From this distribution, the adsorption coefficient can be estimated by fitting an exponential $A\exp(-\eta T/L^3_{box})$ to the blue points. The fitting parameter η (μ m^3/h) gives the adsorption rate in a well-mixed liquid condition, where the overall infection rate per unit volume would be given by η multiplied by densities of bacteria and phages.

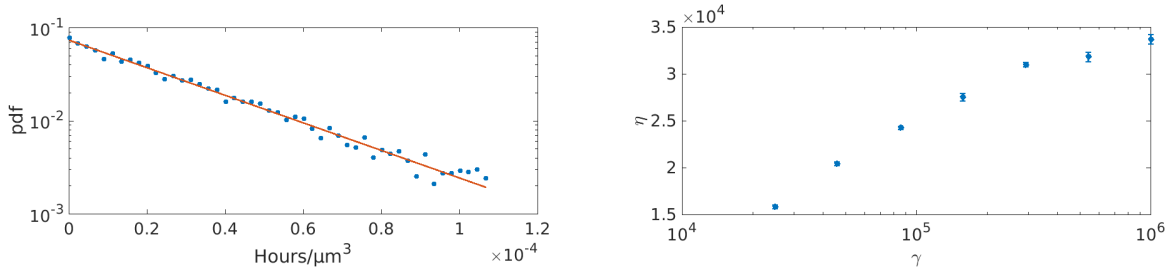

Figure S2: **Left:** The distribution of free phages as a function of T/L^3_{box} (blue points) and fit of $A\exp(-\eta T/L^3_{box})$ (red line). The corresponding adsorption coefficient is $\eta = (3.41 \pm 0.7)\mu m^3/h$. **Right:** The fit-values of η as a function of γ.

This process is repeated for every value of γ, which allows us to map the relationship between the parameters γ and η. The realized relation is shown in Fig. S2 (right).

3 Comparison with explicit modeling of nutrient diffusion and local consumption in microcolony growth

We investigate the accuracy of using the meanfield approximation for nutrient depletion:

$$\dot{n} = -\frac{g(n)}{V}\sum_i \delta_{S_i,0}, \tag{S1}$$

and compare it to the complete field computation. For the complete field computation, we construct a cubic grid for the space: $x_i = i\Delta_{\text{grid}}$, $y_j = j\Delta_{\text{grid}}$ and $z_k = k\Delta_{\text{grid}}$, and divide the time into the discrete points: $t_l = l\Delta T$. For this grid, we define the density of susceptible cells $S(i,j,k,t_l)$, by assigning each cell to the closest grid point. The complete field computation has the continuous form:

$$\dot{n} = -g(n(\vec{x},t)) \cdot S(\vec{x},t) + D_n\nabla^2 n(\vec{x},t). \tag{S2}$$

In our discretized scheme the field equation becomes:

$$\frac{n^{l+1}_{i,j,k} - n^{l}_{i,j,k}}{\Delta T} = -g(n^{l}_{i,j,k}) \cdot S^{l}_{i,j,k} + D_n \hat{\vec{L}} n^{l}_{i,j,k}, \tag{S3}$$

Where the Laplace-operator $\hat{\vec{L}}$ computes the diffusion on the grid. Using the central difference the explicit computation becomes:

$$D_n \hat{\vec{L}} n^{l}_{i,j,k} = \frac{D_n}{\Delta^2_{\text{grid}}}(n^{l}_{i-1,j,k} + n^{l}_{i+1,j,k} + n^{l}_{i,j-1,k} + n^{l}_{i,j+1,k} + n^{l}_{i,j,k-1} + n^{l}_{i,j,k+1} - 6n^{l}_{i,j,k}). \tag{S4}$$

We test the difference between the full field computation and the meanfield computation using the parameters listed in table S1 with two exceptions: $L_{\text{box}} = 50$ μm and $n_0 = 0.1$ μm^{-3}. We observe only minute differences

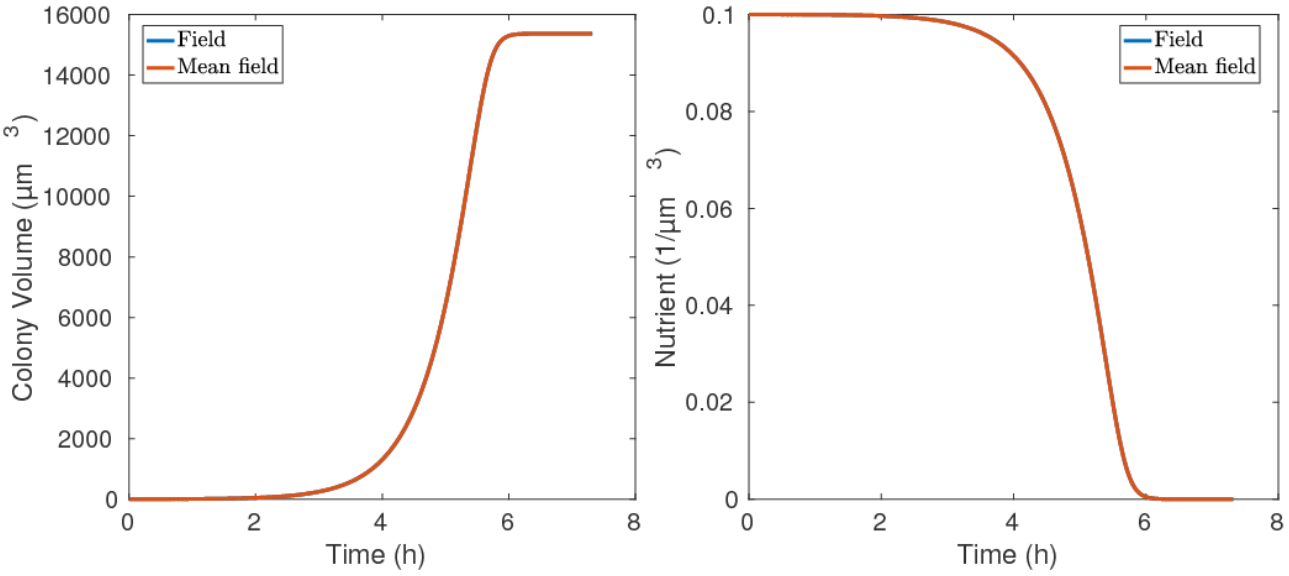

Figure S3: Difference between the full field computation (blue) and the meanfield approximation (red). Because the two curves overlap, the blue curve cannot be visualized in the figure. **Left**. The volume of the colony as a function of time. **Right**. The density of nutrients in the simulation.

between the methods on this scale, but we expect the impact to increase as the total colony volume increases.

4 Measuring the penetration depth from simulations

From the simulations, we observe that the phages can only penetrate the bacterial colony up to a certain depth. This happens because the phages tend to adsorb to the cells close to the surface, which are typically already infected by other phages and get super-infected while they are undergoing latency time before the next phage burst. As a result, the system self-organize to have the cells close to the burst at the surface and the cells in early stage of infection closer towards the core, as seen in Fig. 2 in the main text. By developing a metric to measure this penetration depth, we can observe how the phage parameters influence the phages' ability to invade the colony.

We have operationally measured this penetration depth by first determining the geometric center of the colony $\vec{x}_{gc}(t) = \frac{1}{N}\sum_{i=1}^{N} \vec{x_i}(t)$, where N is the total number of cells in the colony. Next we measure the distance $d_{i,gc}(t)$ from every infected cell to the geometric center as $d_{i,gc}(t) = \|\vec{x}_i(t) - \vec{x}_{gc}(t)\|$. The penetration depth $\Delta R(t)$ is then taken as twice the standard deviation of the distribution of $d_{i,gc}(t)$ at time t.

In Fig. S4, we show a typical example of the penetration depth as a function of time. We notice that the penetration depth initially increases but after approximately 2 hours it stops increasing and reaches a steady state.

In order to plot ΔR for each parameter set in Fig. 3D inset, we converted these time-series into a single measure by cropping the first two hours of each time-series, and taking the mean and standard deviation of the remaining data as the penetration depth and uncertainty, respectively. Since we took an ensemble average of three runs for every parameter set, we took the weighted average over the ensemble and use the standard error of the mean as the error bar in the plot.

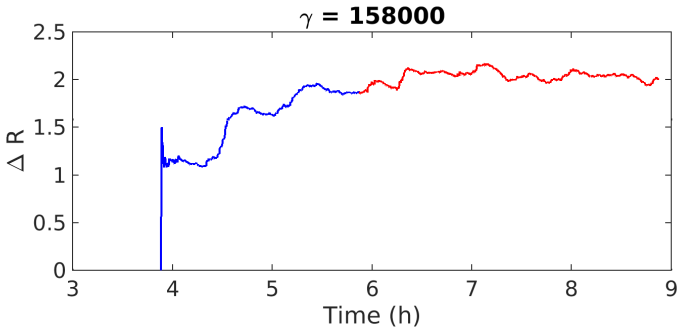

Figure S4: Example of the penetration depth as a function of time. The red section indicates the data used for the steady state measurement. See text for details.

5 Modeling colony growth under phage attack

5.1 Model equation and solution

We model the bacterial microcolony as a solid sphere consisting of an inner core of susceptible cells and a thick outer shell of depth ΔR of infected cells. The model system is illustrated in Fig. S5.

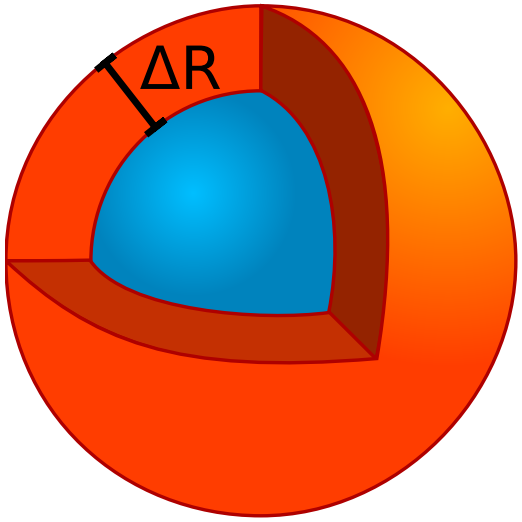

Figure S5: The model system: The cell colony is considered a solid sphere, where the susceptible cells are located in the core (blue), and the infected cells are located as a thick shell (orange) of depth ΔR around the core.

The dynamics of the microcolony can be described by the volume of the core V_O and of the shell $V_{\Delta R}$.

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \frac{\mathrm{d}V_O}{\mathrm{d}t} + \frac{\mathrm{d}V_{\Delta R}}{\mathrm{d}t}$$

The cells are assumed to be in the exponential growth phase and thus the core of uninfected bacteria grows at a rate g and the infected shell is diminished at a rate $rg = 1/\tau_L$ with τ_L being the latency time of phage burst.

$$\frac{\mathrm{d}V}{\mathrm{d}t} = gV_O - rgV_{\Delta R}$$

This expression can be rewritten in terms of the colony radius R.

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \frac{g}{3}\left[(r+1)\frac{(R-\Delta R)^3}{R^2} - rR\right] \tag{S5}$$

This equation allows for stable solutions as long as the growth term is larger than the decay term. There is as such a critical radius R_C, where the derivative is zero:

$$(r+1)(R_C - \Delta R)^3 - rR_C^3 = 0 \tag{S6}$$

The solution of this equation linearized with ΔR is presented in the main text eq. (2). The full solution of eq. (S6) is given by:

$$R_C = \Delta R \left(r^{\frac{1}{3}} (r+1)^{\frac{2}{3}} + r^{\frac{2}{3}} (r+1)^{\frac{1}{3}} + r + 1 \right). \tag{S7}$$

Other than predicting the threshold value of the colony size, (S5) can also be solved by using a few approximations, and then be compared to the volumetric growth curves of the colonies. We expand the first term in eq. (S5) in terms of $\Delta R/R$:

$$\frac{(R-\Delta R)^3}{R^2} = R\left(1 - 3\frac{\Delta R}{R} + \frac{3\Delta R^2}{R^2} - \frac{\Delta R^3}{R^3}\right)$$

and consider solutions of order $\Delta R/R$, where the equations simplify to:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \frac{g}{3}\left[(r+1)(R - 3\Delta R) - rR\right] \tag{S8}$$

The differential equation is a simple first order linear differential equation and can be solved:

$$R(t) = R_0 \cdot \exp\left(\frac{gt}{3}\right) + 3\Delta R \cdot (r+1) \tag{S9}$$

With R_0 as an integration constant, where the radius at time zero is given from eq. (S9) by $R(0) = R_0 + 3\Delta R \cdot (r+1)$. By going only to order ΔR, we can additionally derive a simplified expression of the critical radius as a function of r and g. As before we set the derivative to zero of eq. (S8) to obtain:

$$(r+1)(R_C - 3\Delta R) - rR_C = 0.$$

Which leads to the solution:

$$R_C = 3(1+r)\Delta R.$$

5.2 Growth rate fitting

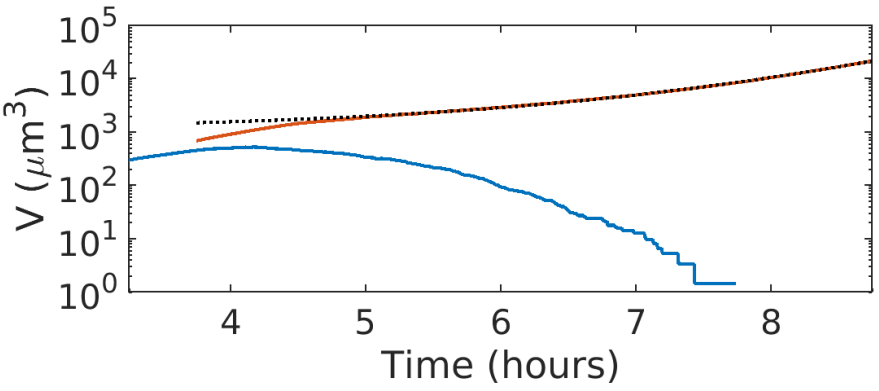

Figure S6: Example of time traces of the colony volume. The blue line is a time-trace from a colony which was just below the survival threshold when the phage attack began. Since the colony is exterminated, a growth rate of zero is assigned. The red line is an example of colony which was just above the survival threshold. The growth rate is determined by fitting of eq. (S9) (black line)

From simulation time-traces, the growth-rate of the colony is fitted to eq. (S9), with R_0 and g being the fitting parameters (Fig. S6 red). When colonies are completely eradicated they are assigned a growth rate of 0 (Fig. S6 blue).

6 Summary of experiments with phage infection of microcolonies at different soft-agar depths

In this section, we summarize the result of two additional repeats of the experiment shown in Fig. 5 in the main text (we call them repeat experiment 1 (exp. 1) and repeat experiment 2 (exp. 2)). The general protocol is given in the Methods section in the main text, with the exception that 3 ml of top agar per plate was used instead of 2.5 ml, and that 250 μl of phage lysate was sprayed onto the plates instead of 500 μl. The phage lysates used in exp. 1 and 2 had concentrations of 4×10^{10} PFU/ml and 3.5×10^{10} PFU/ml, respectively. In addition, in exp. 1, we only prepared one plate for phage spray and one plate for the no-phage control per time point, and the control plates were also used for microscope imaging during the first 8 hours, which means that they were moved from 37°C to room temperature several times during the incubation time.

In both of the repeat experiments, the colony size development was consistent with exponential growth in the first 9 hours, with a fitted doubling time of 29 min and 26 min in exp. 1 and exp. 2, respectively (Fig. S7).

The fraction of visible colonies as a function of pre-incubation time before phage spray is shown in Fig. S8. We see a clear increase in the survival fraction at 6-7 hours, consistent with the result presented in the main text. One notable difference is that in exp. 2, a small number of surviving colonies were observed at all time points, even for the plate sprayed with phage at the 0-hour time point. We noticed, however, that the colonies in the early time-points (especially up to 4 hours) were clustered on one side of the plate, and that the top-agar layer in some cases was noticably thicker on that side of the plate. This suggests that the thicker layer of top-agar provided protection by substantially delaying phage arrival. Therefore, in future experiments, we used the thinnest top agar layer possible (2.5 ml per plate), and care was taken to obtain a homogeneous layer of top agar across the plate.

Finally, Fig. S9 shows the estimate of the radius of final colonies, with and without exposure to phage. The substantial reduction in size as a consequence of phage exposure is seen in both repeat experiments. In exp. 1, the smaller radius from the fluorescence images than from the dark-field images of phage-exposed colonies is evident, suggesting that the surface layer is dead (not producing GFP). In exp. 2, the difference is less pronounced, likely due to additional protection by the thicker top agar.

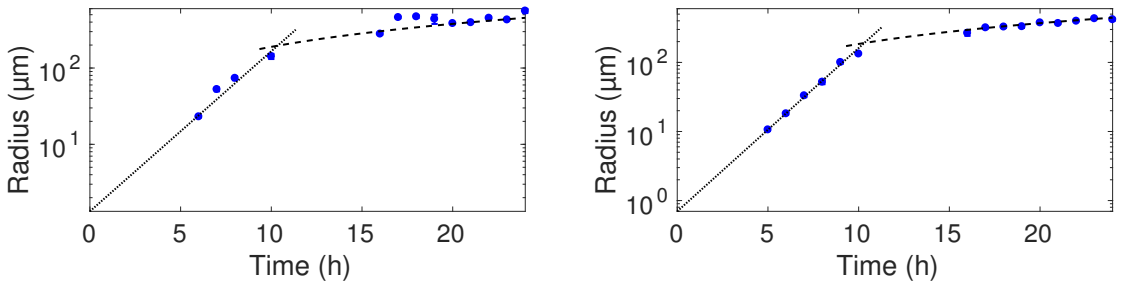

Figure S7: Growth curves for repeat experiment 1 (left) and repeat experiment 2 (right). The fitted doubling time is 28.7 ± 1.2 min and 25.5 ± 0.3 min for exp. 1 and 2, respectively.

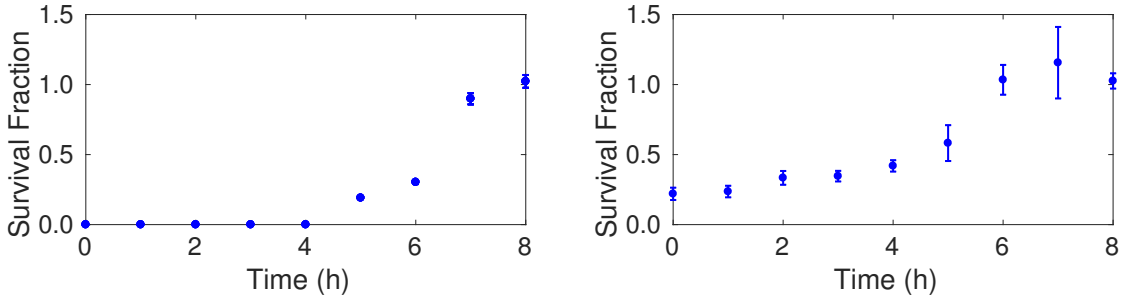

Figure S8: Survival fractions from repeat experiment 1 (left) and repeat experiment 2 (right). The average number of colonies per control plate was 89 ± 4 and 82 ± 4 exp. 1 and 2, respectively.

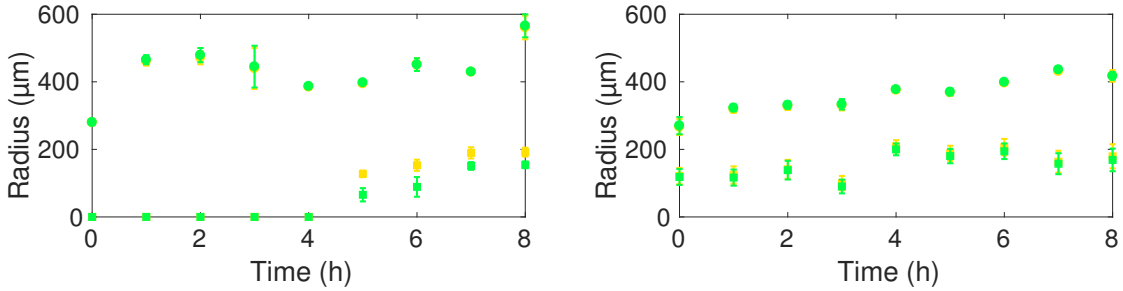

Figure S9: Radii of the final colonies from repeat experiment 1 (left) and repeat experiment 2 (right). The circles show the final colony size in the absence of phage exposure, while the squares show the radii of colonies exposed to phages. The time at which phages were introduced is given on the horizontal axis. Yellow symbols show the radii estimated from dark-field images, while green symbols show the radii estimated from green fluorescence images.

7 Summary of experiments with phage infection of microcolonies with same total growth time

In the experiment shown in Fig. 5, all colonies were incubated for an additional 16 hours following the addition of phages to ensure that the time span allotted for the phages to attack the colonies was uniform across all the plates. As a result, the total incubation time was greater for plates where phages were applied at later time points than for those exposed to phage at early time points. To investigate whether this difference in total incubation time influenced the result, we performed two experiments in which phages were sprayed onto colonies that had been grown for 5, 6 or 7 hours prior to phage exposure, and were then incubated again until they had each reached a total incubation time of 21 hours (set 1) or 23 hours (set 2). We call this repeat experiment 3 (exp. 3). The protocol for exp. 3 was similar to the one described in Methods, with the exception that the phage lysate titer was $\sim 2 \times 10^9$ PFU/ml. For each time point in each set, we had one control plate (no phage added) and one test plate (phage applied at the indicated time point).

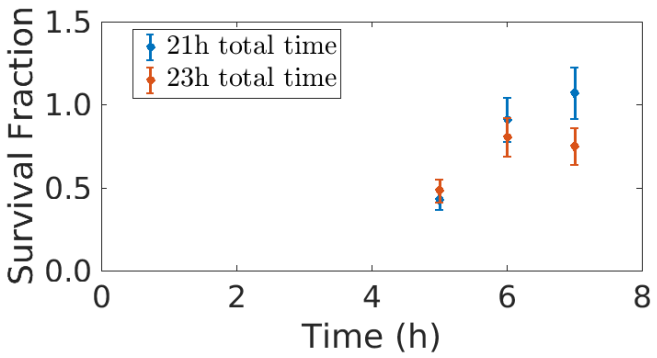

Figure S10: Survival fractions from repeat experiment 3. The average number of colonies per control plate was 19 ± 3. Blue and red data points show the survival fraction of the colonies with 21 or 23 hours of total incubation time, respectively.

Fig. S10 shows the colony survival fraction as a function of incubation time prior to the addition of phage, and Figs. S11 and S12 shows images of several representative colonies from plates sprayed with phages at 5h and 7h, that can be compared to Fig. 4. In conclusion, these experiments confirm that the enhanced survival of colonies sprayed with phages at later time points (shown in Figs. 4 and 5) is not an effect of the longer total incubation time they experienced, but rather an effect of the duration of the incubation time (and therefore colony size) prior to phage addition.

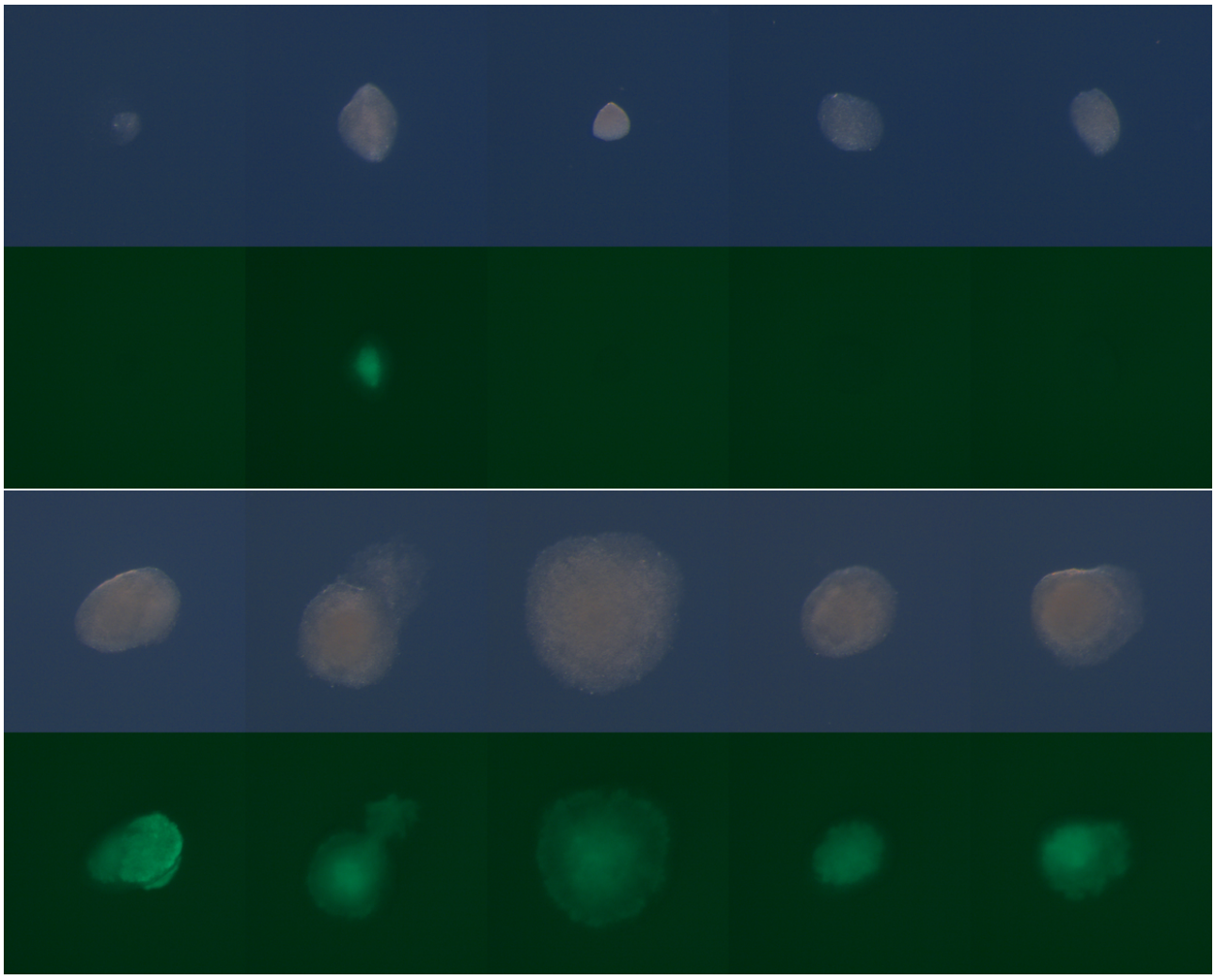

Figure S11: Images of colonies after exposure to phages. These colonies were incubated for 5 hours (top panel) or 7 hours (bottom panel) before being sprayed with phages. The images were captured after additional incubation to reach 21 hours total incubation time.

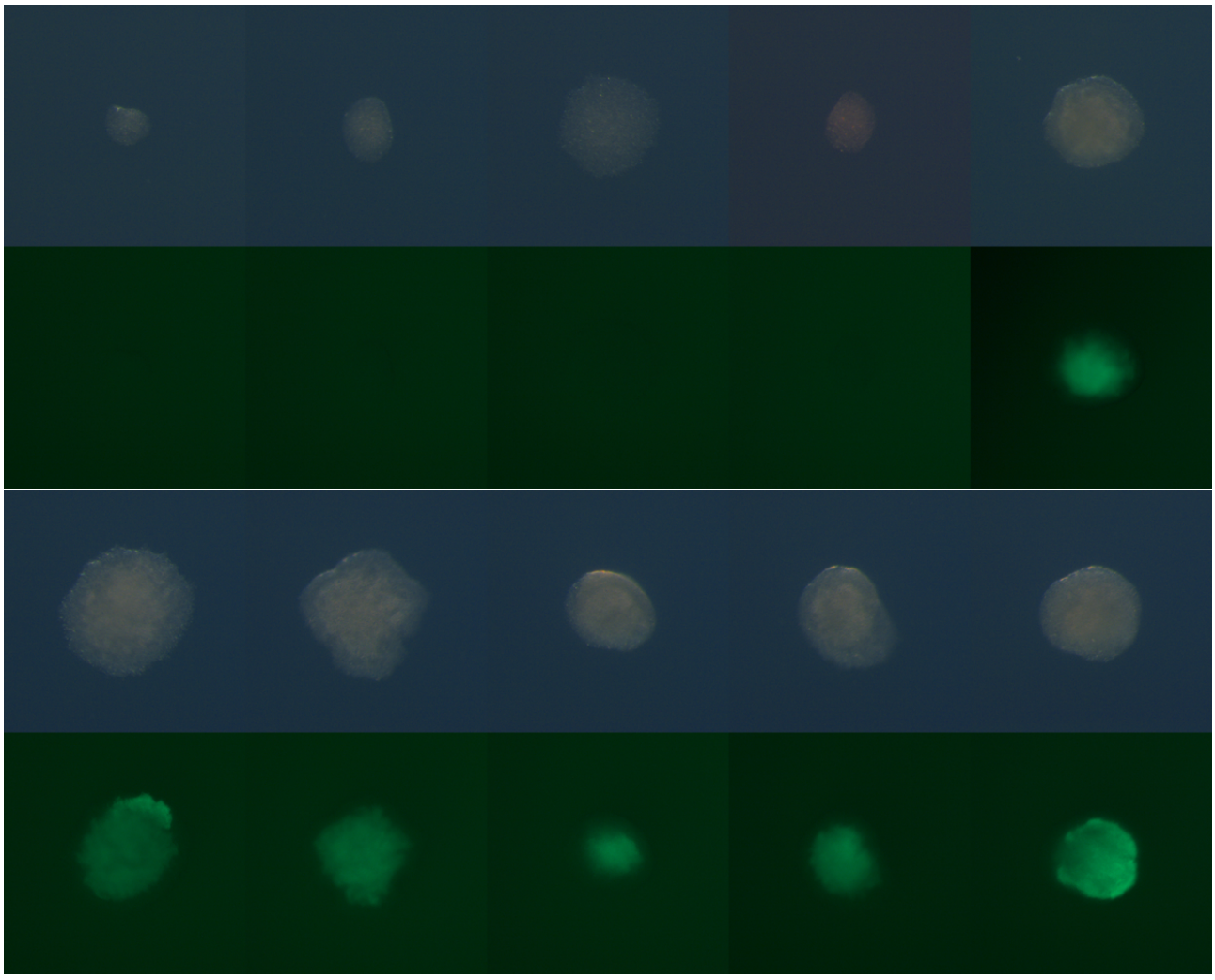

Figure S12: Images of colonies after exposure to phages. These colonies were incubated for 5 hours (top panel) or 7 hours (bottom panel) before being sprayed with phages. The images were captured after additional incubation to reach 23 hours total incubation time.

8 $P1_{vir}$ resistance tests

8.1 Experimental procedures

To determine the level of $P1_{vir}$ resistance among cells in the liquid culture used for seeding the microcolonies, dilutions of the culture in sterile MC buffer were mixed with $P1_{vir}$ at an average phage input >10, incubated for 20 min, and plated on LB plates supplemented with 10 mM MgSO4, 5 mM CaCl2 and 50 μg/ml kanamycin. The frequency of resistant mutants in the culture was calculated as the colony forming units (CFU) per ml of culture treated with phages relative to the CFU per ml of the untreated culture.

To test individual cells in surviving microcolonies from the experiment shown in Fig. 5 for whether they were sensitive or resistant to $P1_{vir}$, 10 individual microcolonies were picked out of the top agar from one plate for each time point of phage application (4-8 hours) and restreaked on individual tryptone plates [5] containing 10 mM sodium citrate to destabilize the phage particles and thereby permit colony formation by both sensitive and resistant cells. 37/50 plates contained *E. coli* colonies after overnight incubation, as shown in Table 1. From these survivors, up to 10 individual colonies were picked from each plate, and cross-streaked against $P1_{vir}$ on R plates (supplemented as in Methods). Since cross-streaks can be difficult to interpret with certainty, any cross-streak where the outcome was unclear was repeated, and, in addition, the outcome of the cross-streak was confirmed for 36 colonies by infection with $P1_{rev6}$ [6] following overnight growth of the colony in shaking liquid culture, and selection for lysogeny on LB plates containing 15 μg/ml chloramphenicol. In every case, the strains that were deemed $P1_{vir}$-sensitive by cross-streak yielded large numbers of chloramphenicol-resistant lysogens, while the strains that were deemed $P1_{vir}$-resistant by cross-streak yielded no or at most a very small number of lysogens, consistent with a small subset of cells having reverted to the P1-sensitive phenotype. Finally, we tested three colonies that were deemed $P1_{vir}$-resistant by cross-streak for their ability to adsorb and propagate $P1_{vir}$ in liquid culture. Specifically, we tested the colonies with id's 5h-3-3, 6h-3-2 and 8h-7-1 from Table S2. At the beginning of the experiment, $P1_{vir}$ were added to diluted liquid cultures containing 1×10^6 CFU/ml to reach an initial phage-to-cell ratio of 0.02. For the following 5.5 hours, the phage content of the mixture was sampled by plaque assay using the double agar overlay method. In the case of the P1-sensitive parent strain SP427, the phage content increased from 3.8×10^4 PFU/ml at the beginning of the experiment to 7.4×10^8 PFU/ml at the last time point. On the contrary, the phage content of the three resistant cultures decreased slightly from $3.8 \times 10^4 \pm 3300$ PFU/ml to $3.0 \times 10^4 \pm 8500$ PFU/ml in the same time span. We conclude that the three tested strains are indeed resistant to infection by $P1_{vir}$, as the phages are unable to propagate on them. They are also refractory to $P1_{vir}$ adsorption, since the number of free phage particles only decreased slightly during the 5.5 hours of growth of the bacterial strains.

8.2 Detailed results of the cross-streak experiment

Table S2 summarizes the detailed results of the $P1_{vir}$ resistance test. As described in the main text, we picked 10 colonies from each phage-sprayed plate that had been pre-incubated for at least 4 hours (shown in the first column), and streaked the colonies on fresh plates containing sodium citrate to inactivate free phage and thereby permit the growth of both sensitive and resistant cells from the original colony. The 10 colonies from each plate are numbered in the second column (Parent colony id #). The third column (# colonies tested) lists the number of "offspring" colonies formed from each parental colony that were tested for phage resistance. When the number is less than 10, it means that less than 10 isolated colonies formed on the fresh plate. When more than 10 colonies grew, we randomly picked 10 for cross-streaking. The fourth column (Resistant), lists the number of colonies that tested $P1_{vir}$ resistant by cross-streaking, while the fifth column (Susceptible) lists the number of colonies that were found to be susceptible to $P1_{vir}$. The sum of the fourth and fifth columns should equal the number in the third column.

Table S2: Full results of the $P1_{vir}$ resistance test.

Time	Parent colony id #	# colonies tested (When less than 10, equal to number of recovered colonies)	Resistant	Susceptible
4h	1	0	-	-
	2	10	3	7
	3	10	0	10
	4	10	0	10
	5	10	0	10
	6	0	-	-
	7	0	-	-
	8	0	-	-
	9	10	10	0
	10	10	0	10
5h	1	0	-	-
	2	1	0	1
	3	10	10	0
	4	0	-	-
	5	0	-	-
	6	0	-	-
	7	0	-	-
	8	10	0	10
	9	1	0	1
	10	10	0	10
6h	1	0	-	-
	2	10	0	10
	3	10	10	0
	4	0	-	-
	5	10	10	0
	6	10	0	10
	7	10	10	0
	8	10	10	0
	9	10	10	0
	10	0	-	-
7h	1	10	10	0
	2	6	5	1
	3	0	-	-
	4	10	10	0
	5	10	0	10
	6	10	0	10
	7	10	0	10
	8	10	10	0
	9	10	10	0
	10	10	0	10
8h	1	10	0	10
	2	10	0	10
	3	10	10	0
	4	10	10	0
	5	10	10	0
	6	10	0	10
	7	10	9	1
	8	10	0	10
	9	10	10	0
	10	10	0	10

9 Effect of the number of phages per bacterium at the time of phage application

In our experimental protocol, we spray the same number of phages onto microcolonies of different sizes, thus the initial average phage input per bacterium (API) decreases for larger microcolony sizes. In order to exclude the possibility that this decrease gives rise to the survival of larger colonies (i.e., protection simply due to a lowered API without any spatial effect), we estimated whether the number of phages applied to the plates would have been sufficient to kill all the phage-sensitive cells had they been in a well-mixed liquid environment rather than a spatially structured environment.

9.1 Host-phage interaction in a well-mixed culture

First, we experimentally measured the change in optical density of bacterial cultures upon exposure to phages at different initial API's in a well-mixed culture. Specifically, 4×10^5 CFU of E. coli SP427 from an overnight culture were subcultured into LB medium supplemented with 10 mM MgSO4, 5 mM CaCl2 and 50 μg/ml kanamycin in each well of a 96-well microtiter plate. Dilutions of $P1_{vir}$ lysate were then added to each well to give the desired API in a total volume of 150 μl. The microtiter plate was incubated at 37°C and shaking at 200 rpm in a Fluostar Omega plate reader, and the optical density of the culture at 600 nm was measured every 5 minutes for 12 hours.

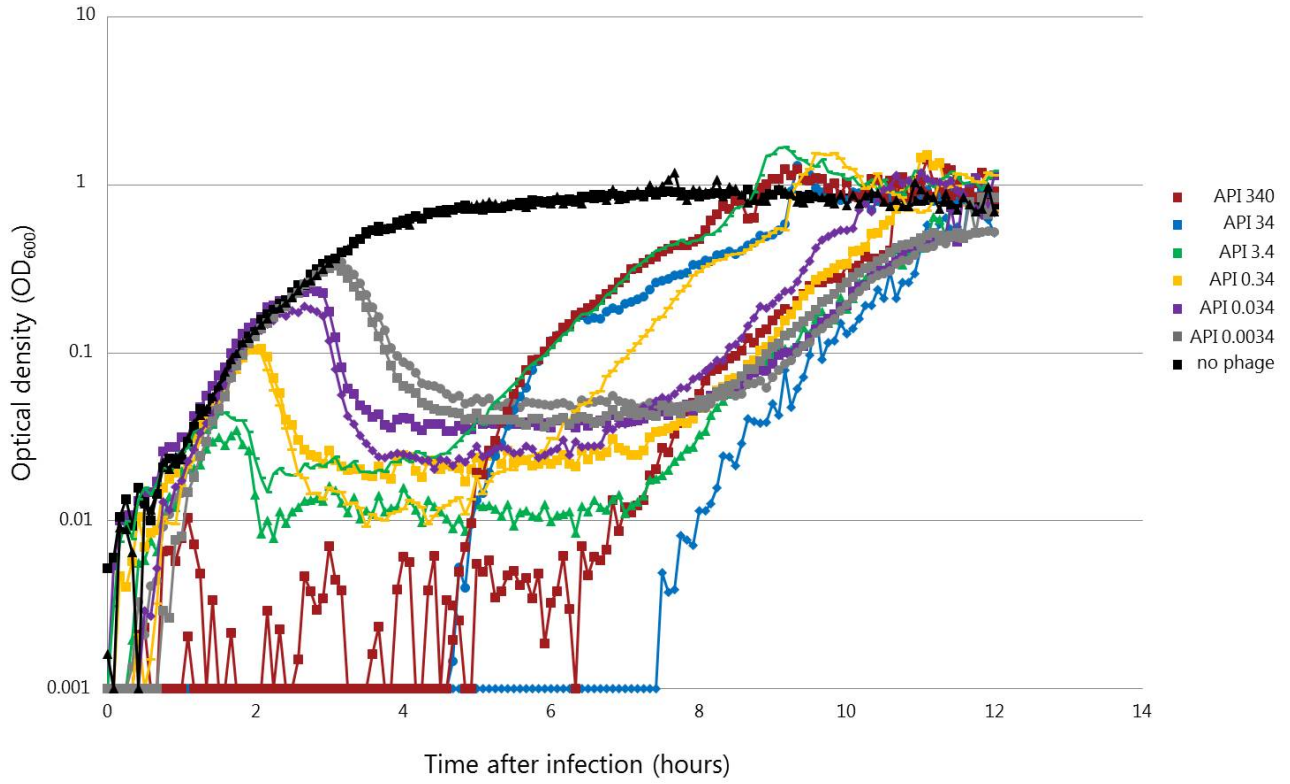

Figure S13: Change of OD_{600} in a well-mixed culture with different initial API's. The values of initial API are displayed in the figure. Two individual measurements are displayed for each value of API. Our measurements of optical density are unreliable at values below 0.01, which explains the large variation in measurements from cultures with very low optical densities.

Fig. S13 shows that for every API, the density of the bacterial population shows a sudden drop after phage addition, but the lag time before the drop occurs increases for smaller API's because the phages need to undergo addtional rounds of multiplication before they are sufficiently numerous to overtake the cell population. Some time after the drop, the OD_{600} increases again and finally plateaues at a similar level as the control cultures where no phage were added (black lines). The second increase is due to the presence of phage-resistant mutants which eventually accumulate. The time at which the resistant mutants accumulate varies between duplicate cultures containing the same initial API, reflecting the stochastic nature of the appearance of resistant mutants [7]. For every tested API, a significant reduction of the host population was observed within 4 hours. From 1h to 4h, the OD_{600} for the no-phage control culture increased about 20-30 fold, corresponding to 4-5 doublings. It is difficult to evaluate the OD_{600} in the first 1h, but evaluating from the later growth rate, at most 1-2 doublings

occurred during that period. This shows that about 6 doubling times after phage encounter gave sufficient time for the phages to kill the hosts even at the low initial API's.

9.2 Evaluation of initial API in the microcolony experiments

We now evaluate whether the amount of phages applied to the plates in the spatially structured microcolony experiment would be enough to eliminate the sensitive hosts had they been in a well-mixed culture. We consider the time point where phages were applied after 7 hours of incubation, at which time all of the colonies survived in the experiment shown in Figs. 4 and 5.

The initial API in our experiment is not straightforward to estimate because of the spatial structure, so we have made two different estimates.

The simplest evaluation of the API is to consider the ratio: (total number of phages sprayed per plate)/(total number of cells per plate). We sprayed 3.5×10^9 phages per plate, and the plates with 7 hours of incubation have about 76 colonies of 10^5 cells per colony. The resulting API$\sim 4.6 \times 10^2$ would certainly be high enough to almost immediately clear the sensitive host population (compare to Fig. S13 at API 340).

However, in reality, many of the applied phages may not have encountered a colony. To take this into account, we now assume that the phages are sprayed uniformly across the surface of the plate ($\sim$ 9 cm in diameter), and we make the very conservative assumption that the colony is only attacked by the phages that land directly on top of it (so that the cross-section of the colony from the top view determines the number of phages that hit it, and the phages do not arrive at the colony by diffusion in the horizontal plane). In this case, the number of phages per colony for the 7h incubation time is estimated to be $\sim 1.5 \times 10^3$, resulting in a lower bound on the initial API of ~ 0.01, which falls within the tested range in Fig. S13 that would have resulted in killing of the phage-sensitive cells after a lag time of less than 6 doubling times. We know that the 7h colonies had nutrients left to support more than 6 doublings after phage exposure, because in the experiment shown in Figs. 4 and 5, the 7h colonies of 30 μm radius had grown to 350 μm in radius in the absence of phages by the end of the experiment, so the volume had increased more than 1000 fold, meaning that the nutrients available after 7 hours supported about 10 additional doublings of the population. Thus, there would have been ample time for the phages to eliminate all the phage-sensitive cells had they been growing in a well-mixed culture. Therefore, we conclude that the reduction of the initial API at increasing microcolony size is not the main determinant of the survival threshold.

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